b) monitoring the modification reaction with a mild and sensitive method such as nondenaturing electrophoresis or electrospray mass spectrometry and optionally confirming the overall structural integrity;

c) protease treatment;

d) mass spectrometry;

e) assaying biological activity of the modified product and optionally assaying stability of the modified product.

95. The method according to claim 94 wherein said proteins or peptides are selected from the group consisting of interleukins, haemopoietic growth factors, peptide hormones, protein hormones, signal peptides and signal proteins.

96. The method according to claim 94 wherein said protein or peptide is selected from the group consisting of the cytokine superfamily, insulin, and prolactin.

97. The method according to claim 96 wherein said protein or peptide is a member of the cytokine superfamily selected from the group consisting of interleukins 1-8, interleukin 10, CM-CSF, TNF, gamma IFN and EPO.

98. The method according to claim 97 wherein said protein or peptide is an interleukin selected from interleukins 1-7.

99. The method according to claim 94 wherein specific digestion with specific proteases and mass spectrometry is carried out for characterisation and localisation of the modified amino acids.

Sub. #2

100. The method according to claim 94 or 99 wherein specific digestion with specific endoproteases and LDMS is carried out for characterisation and localisation of the modified amino acids.

E1

101. The method according to claim 100 wherein said endoprotease is Endo Glu C or Endo Lys C.

102. The method according to claim 94 or 99 wherein the modification is carried out by specific digestion with specific exoproteases and electrospray mass spectrometry is carried out for characterisation and localisation of the modified amino acids.

103. The method of claim 102 wherein the exoprotease is Cathepsine C or carboxypeptidase Y.

Sub. #3

104. The method according to claim 94 or 99, wherein the modification is chemical modification, said modification being alkylation and/or said modification being acylation, such as

acetylation e.g. by Iodo acetate or succinylation, e.g. by succinic anhydride, said modification suitably being a modification under gradually varying conditions, wherein one or more of the following conditions are varied as follows: pH between 5.0 and 7.0, preferably in steps of 0.5 pH units, and/or time or reagent-concentrations are varied.

105. The method according to claim 104, wherein the modification is carried out in the presence of phosphate buffer, preferably in combination with acetic anhydride.

E1

106. The method according to claim 94 or 99 for the introduction of an antagonistic or cell inhibitory activity, wherein the modification has specificity to one or more residues that are involved in catalytic activity.

Sub #4

107. The method according to claim 94 or 99, wherein the modification is within or in close proximity to a metal binding center, preferably a Zinc binding center, suitably said residue is a histidine residue.

108. The method according to claim 94 or 99, wherein the modification is performed by reversibly denaturing the substrate and adding chelating agent to remove the metal ion e.g. in the presence of urea and EDTA, said urea preferably

having a concentration larger than 5 M and said EDTA preferably having a concentration of 50 mM.

Sub. A4

109. The method according to claim 94 or 99, wherein the modification is specific for one type of amino acid, for instance an amine-residue and/or even is specific for only 1 amine-residue in the peptide or protein, said 1 amine for instance being the N-terminus.

E1

110. The method according to claim 94 or 99, wherein the substrate is human interleukin-3, said method preferably providing interleukin 3 modified only at one or more of the following residues: Ala^{1}, His^{26}, Lys^{28}, Lys^{66}, His^{95}, His^{98}, Lys^{100}, or Lys^{116}.

Sub. A5

111. The method according to claim 94 or 99 for the introduction of an antagonistic and/or cell inhibitory activity, said method comprising disruption of phosphate binding.

112. A modified signal substance selected from the group consisting of a protein hormone, peptide hormone, growth factor, a haemopoeitic growth factor, an interferon, an interleukin and a colony stimulating factor with enhanced biological activity, antagonistic activity or cell inhibitory activity, wherein said signal substance contains a

modification within or in close proximity to a catalytic center, preferably such that the catalytic activity is changed, said modification further preferably being within or in close proximity to a metal binding center.

Sub. A5

113. A modified signal substance being a Zinc binding signal peptide, preferably selected from Growth Hormone, prolactin and insulin, the same (cytokine) superfamily as the IL-3 receptor, preferably IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, Epo, IFN-gamma, more preferably selected from the following: IL-2, IL-3, IL-6, IFN-gamma, Growth Hormone, prolactin and insulin, said modified substance having an enhanced biological activity, antagonistic activity and/or cell inhibitory activity, wherein the modification is, preferably within or in close proximity to a Zinc binding center, such that the metal binding properties have been changed.

E1

114. The substance according to claim 113, wherein the metal ion is within or in close proximity to a catalytic center, preferably said metal ion having a catalytic function in the unmodified substance.

115. The substance according to one of claims 112-114, wherein the modification for producing an antagonist is a chemical modification, preferably an alkylation, an acylation

or molecular biological modification like a deletion mutation and/or a substitution mutation.

116. The substance according to one of claims 112-114 wherein the modification is of an amino acid involved in the binding of a metal ion.

117. The substance according to one of claims 112-114 wherein the affinity of the signal substance for the receptor has not decreased by more than a factor of 10.

C1

118. The substance according to one of claims 112-114 wherein the substance is interleukin 3.

Sub. #6

119. The substance according to claim 118, comprising at least one of the following characteristics

a) 0.1 ng of the substance, modified IL-3 inhibits almost 50% of 3ng/ml native IL-3;

b) 3ng/ml of the substance, modified IL-3 suppresses 80-90% thymidine incorporation of 30-100 ng/ml control IL-3;

c) the substance modified IL-3 inhibits control IL-3 by a factor 10-100.

120. The substance according to claim 119 which is human interleukin 3 which has been modified at one or more of the

following residues: Ala^{1}, His^{26}, Lys^{28}, Lys^{66}, His^{95}, His^{98}, Lys^{100}, or Lys^{116}.

121. The substance according to one of claims 112-114, wherein the substance has acquired one of the following combinations of characteristics

a) a decreased stability and increased antagonistic activity for example acetylated IL-3;

b) a decreased stability and increased agonistic activity e.g. N-terminally proteased IL-3 e.g. Cathepsin C treated IL-3;

E1

c) an increased stability and antagonistic activity e.g. succinylated IL-3;

d) an increased stability in combination with an agonistic activity for example C-terminally proteased IL-3 e.g. Carboxypeptidase-Y treated IL-3.

Sub #7

122. A method for preparing a substance according to one of claims 112-114, said method comprising applying a specific chemical modification of selected amino acids to introduce at least one feature selected from the group consisting of enhanced biological activity, enhanced stability, suppressed antigenicity, acquired antagonistic activity, and cell inhibitory activity is introduced into said proteins or peptides, said method comprising the steps of

a) gradual chemical modification of a protein or peptide, followed by

Sub. #7

b) monitoring the modification reaction with a mild and sensitive method such as nondenaturing electrophoresis or electrospray mass spectrometry and optionally confirming the overall structural integrity;

c) protease treatment;

d) mass spectrometry;

E1

e) assaying biological activity of the modified product and optionally assaying stability of the modified product.

123. The substance according to one of claims 112-114 wherein the concentration of substance required for significant inhibition is suitable for clinical application, being less than a hundred fold higher than the native substance concentration, said substance optionally further having increased receptor binding capacity.

124. A method for stimulating stem cell-replication comprising application of a preparation according to claim 118.

Sub. #8

125. A method of obtaining at least inhibition or suppression of a HIV infection wherein the antibody levels are lowered by any of the following steps

a) suppression of antibody production by B-cells, suppression of generation and/or maturation of B-cells, preferably said B cells being anti-HIV-antibody producing B-cells, preferably anti-HIV coat-antibody producing B-cells;

b) plasmaphoresis, partial or complete plasma recovery or selective return of serum,

c) *in vitro* removal of antibodies, preferably HIV-reactive antibodies, preferably HIV-envelope reactive antibodies;

d) *in vivo* depletion, preferably with antibodies, preferably against HIV, preferably against the HIV envelope;

e) leukophoresis.

126. The method according to claim 125, comprising application of a preparation as described by claim 118.

127. The method according to claim 125, comprising application of bi-specific antibodies, preferably directed against the combination CD19/CD3 and/or CD20/CD3.

128. The method according to claim 125 or 126, comprising application of B-cell apoptose inducing substances, preferably APO-1 and/or application of TGF-β as inhibitor of B-cell antibody production.

Sub. G

129. A method for stimulating stem cell-replication comprising application of a preparation according to claim 118 and/or a substance obtainable according to any of the method steps according to claim 94.

Sub. H9

130. A method of gene therapy comprising applying a nucleic acid construct encoding a substance according to one of claims 110-112 to a subject to be treated, said therapy e.g. being directed at HIV infection.

E1

131. A preparation for clinical application comprising a substance according to one of claims 112-114 and an additional signal protein or peptide.

Sub. H10

132. A method for stimulating stem cell-replication comprising application of a substance obtainable according to any of the method steps of claim 94.--

REMARKS

The Examiner indicated that the communication filed on March 22, 1999 in the present application was not complete because the pending claims were not in logical order. The claims have been rewritten in an order which is believed to be satisfactory. No new matter has been added.

It is believed that this is fully responsive to the Communication. If this amendment is not considered to